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Autobiography of Luc Montagnier ☆

I was born in August 18, 1932 in Chabris, France, a town larger than a village but smaller than a city located in Berry, south of the Loire Valley. This was – and still is – a region of agriculture with some renowned products such as welsh rabbits, goat cheeses, and white asparagus. It was the place where my mother had grown up but, in fact, I never lived there.

On my father's side, his parents came from Auvergne, a province of the center of France, made of rich plains and older volcanoes, the later probably being at the origin of my family name: Montagnier, the man living in mountains.

My father had in his youth caught a terrible disease: streptococcus linked arthritis, ending in irreversible lesions in the aortic valve. Therefore, he was declared unfit for military service and had to find a sedentary job: he became an accountant, and excelled in this profession which implied, at that time, mainly handwritten work. He started working in the Poitiers area, and then moved a little farther north in Chatellerault, a small city between Tours and Poitiers.

As an only child, I was cherished by my mother, a housewife, but two events dominated this "pre-war" period, of which I keep a vivid memory:

I was badly hit by a high speed running car when crossing a main road: multiple wounds of which I keep some visible scarves, 2 days in coma, but I emerged from it as if I was born again, at the age of 5 (Fig. 1).

…and 2 years later came the declaration of war in 1939, while all the family was harvesting grapes in the vineyards of my mother's brother. I still remember the images in a newspaper of Warsaw ruins after a bombing by German planes.

And then, in 1940, came the German invasion: my parents and I leaving our house (close to a risky railway station), fleeing on the roads in a little car, and finally more exposed to German bombing during this exodus than if we had stayed home.

The first year of German occupation was terrible, in that we had no food reserves and most of the time, we were starving. I was a rather puny boy and during the 4 years of the war, did not gain a gram! The food substitutes (ersatz) did not stimulate my appetite, when I was dreaming of chocolate and oranges! My father had chronic entero-colitis and, worst, my grandfather (his father) was diagnosed with rectal cancer. He died in 1947 after terrible suffering and each time I visited him, I could see the inexorable progression of the disease. This affected me so much that it is probably one reason why I decided later to study medicine and to start research on cancer.

In June 1944, our house (so close to the railway) was partly destroyed this time by an allied bombing. I keep mixed feelings of this year of liberation of France. It was a great relief, but I also cannot forget the vision of so many killed people, civilians and soldiers, and the images of skinny deportees released from concentration camps. I have hated wars and their atrocities for the rest of my life.

At high school, I was doing well, being usually ahead of my class mates. This is the time at which I became curious of the scientific knowledge, having left behind my religious catholic belief.

Following the example of my father who was tinkering in his leisure days with electric batteries, I set up a chemistry laboratory in the cellar of the new house which was requisitioned to accommodate us. There, I enthusiastically produced hydrogen gas, sweet-smelling aldehydes and nitro compounds (not nitro-glycerine!) that had the unfortunate habit of blowing up in my face.

I was delighted to read – in popularized books – the impressive progress of physics – especially atomic physics. Being good in physics and chemistry – but not as good in maths – I decided not to prepare for the competitions for the "Grandes Ecoles" but instead to register both at the School of Medicine and the Faculty of Sciences of Poitiers. My goal was in fact to start a research carrier in human biology but there was no such specialty in Poitiers, neither in Medicine, nor in Sciences. Since both Faculty and School were at walking distance, I could spend the morning at the hospital and the afternoon attending courses in botany, zoology and geology which were the main disciplines of the License of Sciences.

Fortunately, the new Professor of Botany, Pierre Gavaudan, was a very atypical professor in that his scientific interests were going far beyond the classification of plants. In fact, I owe him to have opened me a large window on what was the beginning of a new Biology, the DNA double helix, the *in vitro* synthesis of proteins, the structure of viruses.

At the same time, I installed in my home a microscope with a time-lapse movie camera, thanks to a gift of my father. This allowed me to do my first research work. I was studying a phenomenon known since 1908 as the phototaxy of chloroplasts: the property of some algae living at the surface of ponds to orient their large unique chloroplast according to the intensity of light; if the light was too intense, the chloroplast turned inside the tubular cell to present its edge. In dark or weaker light, the chloroplast, a flat plate, exposed its larger surface. The phenomenon took a few minutes, which could be analyzed by time-lapse cinematography. Using different glass filters, I could show that it was not the wavelength absorbed by the chlorophyll (red light) which could regulate the orientation of the chloroplasts but indirectly some yellowish pigments absorbing the blue light. I was very proud, at the age of 21, to present this work as a small thesis at the Faculty of Sciences of Poitiers. I was asked by my mentor, Pierre Gavaudan, to write a dissertation also on a literature based subject: it was the L-forms of bacteria. This allowed me to do my first incursion – not the last – in the world of filtering bacteria. I could only find the references on this controversial subject at the library of the Pasteur Institute in Paris. This was indeed the time at which I left Poitiers to Paris,

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Fig. 1.

where I could finish my medical studies, as well as explore some aspects of biology closer to human beings, particularly neuro physiology, virology and oncology.

Having been hired as an assistant at the Sorbonne at the age of 23, I started learning old-fashioned technologies derived from the work of Alexis Carrel on chick embryo heart cultures, as well as that of human cell lines in monolayers. Although my research was not productive at all, I learned from this period a solid expertise of Pasteurian technologies for working in perfectly sterile conditions, without the use of antibiotics.

In 1957, the first description of infectious viral RNA from the Tobacco Mosaic Virus by Fraenkel-Conrat and Gierer and Schramm, determined my vocation: to become a virologist using the modern approach of molecular biology.

I started with the Foot and Mouth Virus in Kingsley Sanders' laboratory at Carshalton near London. Kingsley was an atypical British man, a writer of opera music in his leisure days. He gave me the entire freedom to choose an approach for elucidating the replication of small RNA viruses. I was proud to identify for the first time an infectious double-stranded RNA from cells infected with the murine Encephalomyocarditis Virus, a small single-stranded RNA virus. This demonstrated for the first time that RNA can replicate like DNA, by making a base-paired complementary strand.

In order to complete my knowledge of oncogenic viruses, I moved from Carshalton to Glasgow where a new Institute of Virology had been recently inaugurated, headed by a remarkable virologist, Michael Stocker, and where many high ranking visitors, among them, Renato Dulbecco, were spending sabbatical years.

Working on a small oncogenic DNA virus, polyoma, I could show there, with Ian Macpherson, a new property of transformed cells, that of growing in soft agar. Using this technique, it was easy to detect the transforming capacity of polyoma virus and its DNA. We showed that naked DNA alone carried all the oncogenic potential of the virus. This looks now pretty obvious, but it was not so at that time.

Back to France at the Institute Curie, I extended this finding to a number of cancer cells, transformed or not by oncogenic RNA or DNA viruses. Moreover, this property allowed me to distinguish some *in vitro* steps in the process of transformation which were correlated with some modifications of the plasma membrane and of the carbohydrate layer surrounding it.

A great mystery remained at that time: that of the replication of the oncogenic RNA viruses, now known as retroviruses. Howard Temin (Fig. 2) had proposed the hypothesis of a DNA intermediate, but other possibilities could be considered. I myself tried to find a double-stranded RNA specific of the Rous sarcoma virus, a virus able to infect and transform chick embryo cells. I indeed isolated double-stranded RNA sequences, but they were of cellular origin and existed at the same level in non-infected cells! With Louise Harel, I showed later that this RNA was partly coming from repetitious sequences of DNA. In retrospect, it could, at least in part, represent the recently identified interfering RNAs involved in the negative control of messenger RNA translation.

In 1969–70, the isolation of an RNA-polymerase associated with the viral particles of the vesicular stomatitis virus, led to the idea that perhaps a key-enzyme was also associated with the oncogenic RNA viruses. Indeed, Howard Temin and Mizutani, and independently David Baltimore, discovered in 1970 a specific enzyme associated with Rous sarcoma virus (RSV), the reverse transcriptase (RT), capable to reversely transcribe the viral RNA into DNA.

At about the same time, Hill and Hillova in Villejuif, France, demonstrated that the DNA extracted from RSV transformed cells was infectious and carries the genetic information of the viral RNA, confirming that the enzyme was working faithfully in infected cells.

P. Vigier and I confirmed and extended this discovery by showing that the infectious DNA was associated with the chromosomal DNA of the cells, showing integration of the proviral DNA, as earlier postulated by Temin.

The work on the chicken RSV was extended to similar viruses of mammals, so that many researchers at that time believed that the RT activity was a new highly sensitive tool to detect similar viruses in human leukemia and cancer. This was stimulated by the largely funded virus-cancer program launched by the American NIH. Unfortunately, the hunt for human retroviruses was basically unsuccessful but led to important basic work on the molecular biology of animal retroviruses and the discovery of oncogenes, the "cancer genes".

In 1972, Jacques Monod, then heading the Pasteur Institute, proposed that I create a research Unit in the newly created Department of Virology of the Institute. I accepted the offer and this new laboratory allowed me to develop new avenues of research within the general theme of Viral Oncology with the ultimate goal remaining the detection of virus involved in human cancers.

Thus, I became interested in the mechanism of action of interferon and its role in its expression of retroviruses. I came in this field after

Fig. 2.

having demonstrated the biological activity of interferon messenger RNA in collaboration with two world-known experts in the field, Edward and Jacqueline De Maeyer.

From 1973 and on, Ara Hovanessian and his co-workers joined my Unit and added a new dimension on the subject: the complex biochemical mechanism sustaining the antiviral activity of this remarkable group of cellular proteins.

In 1975, two other researchers joined my Unit and brought their expertise on murine retroviruses: it was Jean-Claude Chermann and his collaborator, Françoise Barré-Sinoussi (Fig. 3). The latter had mastered the detection of retroviruses by their RT activity. I convinced them to participate in a joint study inside the Unit to look again for retroviruses in human cancers. We started in 1977 with blood samples coming from different Parisian Hospitals and biopsy specimens.

Two advances made in other laboratories boosted this search:

In Villejuif, France, Ion Gresser had prepared a potent antiserum neutralizing any molecule of alpha endogenous interferon produced by individual cells. This interferon, we realized, was produced by mouse cells induced to express some of their endogenous retroviruses. Its blockade by the antiserum increased by up to 50 times the production of endogenous retroviruses in the culture medium. We could conclude that, despite the fact that endogenous retroviruses are integrated in the genome of vertebrates for millions of years, their expression is still controlled by the interferon system, like that of exogenous viruses!

At about the same period, the discovery by Doris Morgan and Frank Ruscetti in Dr Gallo's laboratory of a growth factor allowing the *in vitro* multiplication of human T lymphocytes (TCGF, then named interleukin 2, IL-2) made it possible to propagate T lymphocytes in sustained cultures.

We knew at that time that some retroviruses involved in mouse mammary tumor formation (MMTV) could not only be expressed in the tumor cells but also in the circulating lymphocytes.

Taking advantage of these two advances, we started a search for retroviruses in human cancers, using anti-interferon serum and IL-2, with particular focus on the T lymphocyte cultures from breast cancer patients.

Indeed, in 1980, we were able to detect a DNA sequence close to that MMTV not only in the cells of an inflammatory breast cancer (from a North African woman), but also in her cultured T lymphocytes. A second patient showed similar results.

Unfortunately, the molecular tools we had at that time could not tell us whether we were dealing with endogenous retroviral sequences or with an exogenous virus. Nowadays, having in hands more powerful technologies, I am planning to reinitiate these studies.

But in 1983, the same approach, the use of anti-interferon serum, and the use of long-term cultures of T lymphocytes greatly facilitated the isolation of HIV.

My involvement in AIDS began in 1982, when the information circulated that a transmissible agent – possibly a virus – could be at the origin of this new mysterious disease. There were at that time only a few cases in France, but they attracted the interest of a group of young clinicians and immunologists. They were looking for virologists, especially retro-virologists, as a likely hypothesis was that HTLV – the only human retrovirus known so far, recently described by R.C. Gallo – could be involved. Retroviruses causing leukemia in rodents often cause also a wasting syndrome which could be the result of secondary immune depression. This was also the case of patients suffering from leukemia induced by HTLV.

A member of the working group, Françoise Brun-Vézinet, was a former student of the Virology Course I was then directing. She called me up to organize the search of the putative retrovirus from a patient presenting with an early sign of the disease, lymphodenopathy. The patient was a young gay man who had been traveling to the USA, who was consulting Dr. Willy Rozenbaum – one of the leaders of the working group – for a swollen lymph node in the neck. The gland was not painful; it was just for the patient a question of aesthetics.

The reasoning was that if we were to find a virus at this early stage of the disease, it could be more a cause than a consequence of the immune depression.

Another incentive to start this research was a request from the producers of hepatitis B virus vaccine in the industrial subsidiary of the Pasteur Institute. They were using plasma from American blood donors and were concerned by the risk of transmission of the AIDS agent through their procedure of viral antigen purification.

The lymph node biopsy arrived on January 3, 1983, a date which I remember well because it was also the first day of the Virology Course at the Pasteur Institute which I had to introduce. I could dissect the small hard piece only at the end of the day. I dissociated the lymphocytes with a Dounce glass homogenizer and started their stimulation in culture with a bacterial mitogen, Protein A, known as an activator of B and T lymphocytes, since I did not know which fraction of lymphocytes could produce the putative virus. Three days later, I added the T cell growth factor I had obtained from a colleague working in the laboratory of Jean Dausset.

Fig. 3.

The T cells grew well. As previously established in a protocol for the search of retrovirus in human cancers, it was decided with my associates, Françoise Barre-Sinoussi and Jean-Claude Chermann, to measure the RT activity in the culture medium every 3 days. In day 15, Françoise showed me a hint of positivity (incorporation of radioactive thymidine in polymeric DNA), which was confirmed on the following week.

We had evidence of a retrovirus, but this was just the beginning of a series of questions:

Was it close to HTLV or not?

Was it a passenger virus or, on the contrary, the real cause of the disease?

In order to answer these basic questions, we had to characterize the virus biochemically and immunologicaly, and for that, we needed to propagate it in sufficient amounts. Fortunately, the virus could be easily propagated in activated T lymphocytes from adult blood donors. No cytopathic effect was observed with this first isolate, but unlike HTLV infected cultures, no transformed immortalized cell lines could emerge from the cultures, which always died after 3–4 weeks as do normal lymphocytes.

By contrast, subsequent isolates I made from culture of lymphocytes of sick patients with AIDS were cytopathic for T lymphocytes culture and – we discovered later – could be grown up in larger amounts in tumor cell lines derived from leukemia.

Shortly after the virus isolation, my co-workers and I could show that it was not immunologicaly related to HTLV, and in electron microscopy, it was very different from HTLV viral particles. In fact, as soon as June 1983, I noticed the quasi identity of our virus with the published electron microscopy pictures of the visna virus of sheep, the infectious anemia virus of horse, the bovine lymphocytic virus: it was a retrolentivirus, a subfamily of viruses causing in animals long lasting disease without immunodeficiency.

This indicated clearly that we were dealing with a virus very different from HTLV, and my task was now to organize a team of researchers to accumulate proofs that this new virus was indeed the cause of AIDS.

It was an exciting period since every Saturday morning, when we had a meeting in my office, new data were brought by my associates favoring the causative role of the virus. The viral isolates were called LAV, for Lymphadenopathy Associated Virus, when they were isolated from patients displaying swollen lymph nodes, a frequent sign of the early phase of infection. The isolates made from patients with full blown AIDS were called Immuno Deficiency Associated Viruses (IDAV). The latter generally grew better in T lymphocyte culture and induced the formation of large syncitia, resulting from the fusion between several infected cells. Some of them – we found it later – could also multiply in continuous cell lines of B or T cell origin. The later property greatly facilitated the massive production of the virus for commercial use.

By September 1983, I could make a synthetic presentation of all our data favoring a causal link between the virus and the disease at a meeting on the HTLV organized by L. Gross and R. Gallo at Cold Spring Harbor.

This presentation was received with skepticism by a scarce audience (it was a late night session) and the HTLV theory was still prevailing. Mentally, most attendants were not prepared to accept the idea of a second family of retroviruses (lentiretroviruses) existing in humans and causing immune deficiency, and having no counterpart in animals!

This situation is not infrequent in science as new discoveries often raised controversy. The only problem is that it was a matter of life and death for blood transfused people and hemophiliacs, since a serologic blood test using our virus antigens was already working at the laboratory scale, but awaited industrial and commercial developments.

This came in 1985, after two other teams of researchers – first that of Dr Gallo at NIH in early 1984 and that of Jay Levy in San Francisco – confirmed and extended our findings. In particular, Dr Gallo and his associates gave more strength to the correlation between the virus and the disease, improved the detection of the antibody response and could grow several viral strains, including ours, in T cell lines of cancer origin. Meanwhile, my co-workers showed the tropism of the virus for CD4+ cells and identified the CD4 surface molecule as the main receptor to the virus.

The rest of the story is described in the next chapter. I would just like to illustrate how I discovered what I believe are two important phenomena for explaining the destruction of the immune system induced by HIV.

During the latent phase of the infection, no virus is found in the blood, it is mostly localized in lymphocytes of lymphatic tissues and yet, we found that most of the lymphocytes present in the blood are sick! In 1987, a young visitor from Sweden, Jan Alberts, came to my lab. He wanted to cultivate human lymphocytes in serum-free synthetic medium and to learn some technologies about HIV culture. The surprise came when we compared in his medium the viability of lymphocytes from healthy donors and those from HIV infected patients, even in their early-asymptomatic-stage of infection. While the former could survive several days without dying, the majority (more than 50%) of the latter died very quickly. Addition of interleukin 2 partially prevented their death.

When we used normal culture medium supplemented with fetal calf serum, the same difference was observed, although the survival time of the lymphocytes from infected patients was longer.

It took not very long before three of my collaborators found the reason of such deaths: apoptosis. This is an active process by which the cell “decides” to die in a clean way, without releasing too much of toxic compounds into the medium.

It is a physiological way to prevent abnormal proliferation of activated lymphocytes clones, but here the phenomenon was enormous and occurred not only on the main cellular targets of HIV infection, CD4+ T lymphocytes, but also on cells which were not infectable by the virus, such as CD8+ T lymphocytes, B-lymphocytes, monocytes, natural killer cells…. Clearly, it was a general phenomenon, the culture simply revealing a predisposition to apoptosis of the majority of circulating blood cells, although most of them were neither infected nor infectable. Indeed, my collaborator, Marie-Lise Gougeon, found a very good relation between *in vitro* apoptosis and the *in vivo* observed drop of CD4 T cells in patients.

We have spent a lot of time trying to find the origin of this massive apoptosis, without finding a completely satisfactory explanation: the most likely is the intensive oxidative stress existing in patients since the beginning of their infection. This is also a finding I am very proud of: although oxidative stress has been – and still is – completely overlooked by AIDS researchers, it is likely to aggravate the wrong activation of the immune system at the origin of its decline, and also to trigger inflammation through the production of cytokines.

Of course, the next question arises: what are the factors causing oxidative stress; viral proteins, fragments of viral DNA, co-infection with mycoplasmas? Even after 25 years, we still do not know the complete answer. But the phenomenon does exist and needs to be treated, while most AIDS clinicians do not care of it at all!

The treatment by combined antiretroviral therapy has, without doubt, changed the prognosis of this lethal disease, from death condemnation to an almost “normal” life. However, the virus is still there, ready to multiply when the treatment is interrupted, and not all HIV infected patients of the developing world have access to it! And the epidemics still kill 2–3 millions of people each year. There is therefore an absolute necessity to resolve these problems and basic research, as well as clinical research, has to be continued.

In addition, I realized in the 1990s that research should not only be localized in the wealthy laboratories of the developed countries, but

also in Southern countries in which a lot of patients were suffering of AIDS and many other diseases like tuberculosis and malaria.

Too many examples showed that collaboration between Northern and Southern research laboratories is unequal, the South providing serum samples to be analyzed in the North. This "safari" concept is wrong. There are now many young researchers trained in Northern laboratories who would like to come back to their own countries, but are prevented to do so because laboratories and adequate structures are missing. Moreover, one has to be on the regions where disease proliferates to realize how complex is the reality.

This is why I initiated with the former Director General of UNESCO, Federico Mayor, a Foundation aimed at creating in African countries Centres for Research and Prevention. Although the task was difficult, this concept met with enthusiastic colleagues and medical doctors and also found the support of local governments, particularly in Ivory Coast and Cameroun.

I wish that from these pilot experiments, a whole network of similar Centers could cover all the countries of the developing world where the populations are badly hit by the epidemics.

Another lesson I drew from my AIDS experience was the weakening effect of oxidative stress on the immune system and its pro-inflammatory role in many chronic diseases, such as Parkinson, Alzheimer, and rheumatoid arthritis; a likely consequence of chronic infections? Or both consequence and cause? Many questions which can be resolved only by hard work and innovative thinking… I hope to be able to continue both.

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